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SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/906,689	06/30/92	MURRAY	M 3176-1

EXAMINER
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27

ART UNIT	PAPER NUMBER
1803	2

DATE MAILED: 08/25/92

10-25-92
11-25-92
1-25-93
2-25-93

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 6/30/92 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), — days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-8 are pending in the application.

   Of the above, claims _____ are withdrawn from consideration.

2. ☐ Claims _____ have been cancelled.

3. ☐ Claims _____ are allowed.

4. ☒ Claims 1-8 are rejected.

5. ☐ Claims _____ are objected to.

6. ☐ Claims _____ are subject to restriction or election requirement.

7. ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).

EXHIBIT F

11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ [illegible] (see explanation).

12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____ ; filed on _____

This application has been filed with informal drawings which are acceptable for examination purposes only. Formal drawings will be required when the application is allowed. The required changes to the drawings are detailed on the accompanying PTO Form 948.

Claims 1 - 8 are rejected under 35 U.S.C. 101 because the claimed invention lacks patentable utility.

The specifications provide data only for inhibiting HIV in cells in culture. There is no data to substantiate the alleged utility for treating human subjects infected with HIV. There is no detailed regimen or protocol defined that includes dosage, required blood levels, period of treatment, etc. Without statistically significant data documenting the claimed method for treating patients, the person of ordinary skill in the art, knowing the unpredictability of extrapolating from in vitro results to in vivo performance, would have good reason to doubt efficacy of applicant's invention. The burden falls on the applicant to substantiate his alleged in vivo method of treating humans with nicotinamide.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

EXHIBIT F

The specification is objected to under 35 U.S.C. 112, first paragraph, as failing to provide an adequate written description and failing to teach adequately how to make and/or use the invention, i.e. failing to provide an enabling disclosure.

The specifications provide data only for inhibiting HIV in cells in culture. There is no data to substantiate the alleged utility for treating human subjects infected with HIV. There is no detailed regimen or protocol defined that includes dosage, required blood levels, period of treatment, etc. Without statistically significant data documenting the claimed method for treating patients, the person of ordinary skill in the art, knowing the unpredictability of extrapolating from in vitro results to in vivo performance, would have good reason to doubt efficacy of applicant's invention. The burden falls on the applicant to substantiate his alleged in vivo method of treating humans with nicotinamide.

Claims 1 and 7 read on a method of treating humans infected with HIV with "a post transcriptional inhibitor of HIV". These claims encompass alleged inhibitors of HIV that the specifications do not even identify by chemical name. Since the method of treatment using nicotinamide is not enabled, certainly these other unnamed agents without even so much as in vitro data are also not enabled.

Claims 1 - 8 are rejected under 35 U.S.C. 112, first

paragraph, for the reasons set forth in the objection to the specification.

Claim 4 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The meaning of " 3 D " should be replaced by the three specific symptoms symbolized by this term.

The Kull, Jr. et al. and White et al. references are cited in order to establish the contemporary knowledge in the art of nicotinamide as a important dietary consitutent and as a wound healing constituent.

No claim is allowed.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 308-4227.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Kunz whose telephone number is (703) 308-3995.

G.K.

Gary L. Kunz:glk
August 24, 1992

JOHNNIE R. BROWN
SUPERVISORY PATENT EXAMINER
ART UNIT 183

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Michael F. Murray, M.D.

Serial No.: not yet known

Filing Date: submitted herewith

For: HUMAN IMMUNODEFICIENCY VIRUS (HIV)

CERTIFICATE OF EXPRESS MAIL

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

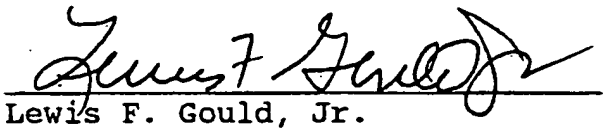
Sir:

I hereby certify that this document, namely the above-identified complete patent application is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated below and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

"Express Mail" mailing label number TB135927132.

Date of Deposit June 30, 1992.

Respectfully submitted,



Lewis F. Gould, Jr.
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Philadelphia, PA 19103
(215) 575-6000

Docket # 3176-1

PATENT APPLICATION TRANSMITTAL LETTER

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS Docket No. 3176-1

Transmitted herewith for filing of the patent application of

Michael F. Murray, M.D.

for METHOD OF INHIBITING HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Enclosed are:

5 sheets of drawing (3 sets of photocopies)
____ an assignment of the invention to _____
____ a certified copy of a _____ application
____ associate power of attorney
X verified statement to establish small entity status under 37 C.F.R. 1.9 and 1.27 (Independent Inventor)
X Certificate of Express Mail

CLAIMS AS FILED			SMALL ENTITY		Other than a Small Entity	
FOR	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
Basic fee				$ 345		$ 690
Total Claims	8 -20-	0	X $10 =	$	X $20 =	$
Indep Claims	3 -3-	0	X $36 =	$	X $72 =	$
multiple dependent claim present			X $110=	$	X $220 =	$
			TOTAL	$ 345	TOTAL	$

If the difference in Col. 1 is less than zero, enter "0" in Col. 2

____ Please charge my Deposit Account No. _____ in the amount of $_____.
____ A duplicate of this sheet is enclosed.
X A check in the amount of $ 345.00 to cover the filing fee is enclosed.
X The Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 19-4235. A duplicate copy of this sheet is enclosed.
X Any additional filing fees required under 37 C.F.R. 1.16.
X Any patent application processing fees under 37 C.F.R. 1.17.

June 30, 1992
Date

Lewis F. Gould, Jr.
Registration No. 25,057

DECLARATION FOR PATENT APPLICATION

Docket: 3176-1

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD OF INHIBITING HUMAN IMMUNODEFICIENCY VIRUS (HIV), the specification of which

(check one) X is attached hereto.
___ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all known information which is material to patentability as defined in Title 37, Code of Federal Regulation Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
			Yes	No
(Number)	(Country)	(Day/Month/Year Filed)		
			Yes	No
(Number)	(Country)	(Day/Month/Year Filed)		
			Yes	No
(Number)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Appln. Serial No.) (Filing Date) (Status-patent, pending, abandoned)

(Appln. Serial No.) (Filing Date) (Status-patent, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Walter J. Blenko, Jr., Registration No. 18,526; Karl L. Spivak, Registration No. 18,934; Arnold B. Silverman, Registration No. 22,614; Richard V. Westerhoff, Registration No. 24,454; Lewis F. Gould, Jr., Registration No. 25,057; Stephan P. Gribok, Registration No. 29,643; Robert E. Greenstien, Registration No. 27,556; Suzanne Kikel, Registration No. 28,230; Michael J. Kline, Registration No. 31,632; Craig G. Cochenour, Registration No. 33,666; John V. Silverio, Registration No. 34,014; Robert J. Kapalka, Registration No. 34,198 and George Stacey, Registration No. 35,688.

Please direct all correspondence to: Lewis F. Gould, Jr. Eckert Seamans Cherin & Mellott, Suite 3232, 1700 Market Street, Philadelphia, Pennsylvania 19103, (215) 575-6000.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Michael F. Murray, M.D.
Inventor's signature Michael F. Murray, M.D. Date 6/30/92
Residence Philadelphia, PA 19154
Citizenship USA
Post Office Address 3867 Ronnald Drive
Philadelphia, PA 19154

Full name of second joint inventor, if any _____
Second Inventor's signature _____ Date _____
Residence _____
Citizenship _____
Post Office Address _____

METHOD OF INHIBITING HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Background of the Invention

1. Field of the Invention:

This invention relates to the inhibition of human immunodeficiency virus (HIV) replication, the etiological agent clinically associated with acquired immunodeficiency syndrome (AIDS). More particularly, the invention relates to administering a therapeutically effective amount of niacin, such as nicotinamide, to host cells that are infected with HIV as well as those not infected.

2. Prior Art:

AIDS is the clinical syndrome associated with HIV infection. AIDS already has claimed nearly 300,000 lives. HIV is believed to have infected approximately 10 million people around the world and 40 million people are expected to be infected by the end of the century. In the little more than ten years since the first reported cases of AIDS, a great deal has been learned about this retroviral disease and its diverse manifestations. There remain, however, a number of clinical expressions of AIDS which go unexplained despite the efforts of the medical community to elucidate their etiology.

Viruses, such as HIV, are packets of infectious nucleic acids, the genetic material, surrounded by a protective protein coat. Viruses are unable to generate metabolic energy or to synthesize proteins and thus are characterized by total dependence on living cells for replication and proliferation. It has been discovered that HIV contains the enzyme RNA-

EXHIBIT F

directed DNA polymerase or reverse transcriptase which is required for the synthesis of viral DNA in a living cell and is crucial for HIV replication.

Medical researchers in the past have focused their efforts on the development of anti retroviral agents which inhibit or block reverse transcriptase (RT) activity. Such agents include among others, AZT (3'-azide-3'-deoxythymidine), DDI (2'-3'-dideoxyinosine), and DDC (2'-3'-dideoxycytidine), each of which are thought to block HIV proliferation in cells. However, these RT inhibitors do not cure HIV, do not block HIV replication completely, and they frequently produce undesired side effects.

Other studies have focused on inhibiting protein synthesis in HIV infected cells in a manner which result in killing of those cells. For example, U.S. Patent No. 4,867,976 discloses a method of treatment of HIV using a liposome containing a diphtheria toxin dissociation fragment to specifically inhibit protein synthesis in an HIV infected cell. The diphtheria toxin induces covalent modification and inactivation of the elongation factor necessary for elongation of a peptide chain. The catalyzed reaction involves the cell's nicotinamide adenine dinucleotide (NAD^+) donating its adenosine diphosphate (ADP) ribose moiety (ADP-Ribose) to the elongation factor with the release of nicotinamide.

EXHIBIT F

Another study of suppression of HIV replication at the transcriptional activation level is Pauling et al., Proc. Natl. Acad. Sci 87:7245, 1990, which discloses that ascorbic acid (vitamin C) is effective to suppress HIV replication in vitro by diminishing viral protein production in infected cells and RT stability in extracellular virons.

Niacin, a component of Vitamin B complex, is a generic term that can apply to both nicotinic acid, i.e., $C_6H_5NO_2$, (pyridine-3-carboxcylic acid) or nicotinamide, i.e., $C_6H_6ON_2$ (3-pyridinecarboxamide). Niacin is a precursor to the biosynthesis of nicotinamide adenine dinucleotide (NAD). Nicotinamide adenine dinucleotide participates in a wide array of oxidation-reduction reactions catalyzed by dehydrogenase or oxido-reductase enzymes. Virtually every aspect of cellular metabolism involves NAD/NADH or NADP/NADPH dependent reactions. In absence of sufficient supplies of NAD or niacin precursors for NAD biosynthesis, cellular functions and life itself would be impaired. (DiPalma et al., Annu. Rev. Nutr. 11:169, 1991). It has not been suggested heretofore to use niacin as an HIV inhibitor.

Nicotinamide, the amide of nicotinic acid, is produced in cells as an end product of a number of mono and poly ADP reactions. The reactions are characterized by:

NAD → ADP-Ribose-Protein + Nicotinamide.

This ADP-Ribosylation reaction has the potential to deplete cellular NAD. Nicotinamide is a known end-product and inhibitor of ADP ribosylation reactions.

Since only the intact virus can infect a cell, it is desired to inhibit intact and infective HIV replication. It has not been suggested heretofore that suppression of intact and infective HIV gene expression in host cells of patients infected with HIV can be accomplished with nicotinamide. The present invention provides a method for inhibition of HIV replication in cells infected with the intact virus through administration of a therapeutically effective amount of nicotinamide. The present invention is based on the inventor's clinical observations, knowledge of compound toxicity in humans, and observation of intact virus inhibition.

Pellagra is a disease which was first described in 1735. Through the work of Elvehjem et al., J. Am. Chem. Soc. 68:1767, 1937, it became clear that endemic pellagra was caused by nicotinic acid (niacin) deficiency, a precursor to NAD biosynthesis. Classically, the disease was clinically identified by dermatitis, diarrhea, and dementia (3 D's). Nicotinamide replacement has been used to treat pellagra. Furthermore, Nicotinamide, a B complex vitamin, is relatively nontoxic when administered to humans even in substantial quantities, i.e., of up to as much as 5 grams per day.

Clinical observation shows that both pellagrins and AIDS patients demonstrated like symptoms (i.e., the 3 D's). This observation led to the idea that it could be possible to inhibit HIV by administering a therapeutically effective amount of nicotinamide to patients infected with HIV. Surprisingly, it was found that HIV replication is inhibited by administration of nicotinamide in a therapeutically effective dose. The mechanism through which this inhibition occurs is not necessarily understood, although it is theorized that nicotinamide, administered in therapeutically sufficient quantity, may function as an ADP ribosylation inhibitor which serves to suppress HIV gene expression at the post transcriptional level.

SUMMARY OF THE INVENTION

It is the object of the invention to inhibit HIV replication and proliferation through administration of a post transcriptional inhibition of HIV.

It is another object of the invention to inhibit adverse effects associated with HIV production.

It is still another object of the invention to inhibit HIV with relatively non-toxic inhibiting agents.

It is another object of the invention to inhibit HIV production by combining the method of this invention with known HIV inhibitors, such as AZT, DDI, DDC, etc.

These and other objects of the invention are achieved by providing a method of administering a therapeutically effective amount of nicotinamide to a patient infected with HIV, the etiological agent clinically associated with AIDS.

The invention is grounded on the observed similarities of clinical expressions of AIDS and pellagrins patients. Prominent among unexplained infirmities exhibited by AIDS patients are seborrheic dermatitis, AIDS enteropathy and AIDS dementia. The inventor's recognition of the striking common symptoms presented by the three D's in pellagra and AIDS patients, led to a possible alternative explanation for some idiopathic AIDS symptoms. The inventor believed and concluded that HIV induces a pellagra-like state through metabolic depletion of niacin, and that the pellagrin like symptoms did

not arise through dietary deficiency. The inventor further believes that HIV replication requires the NAD hydrolysis reaction, i.e., ADP ribosylation, during the post transcription portion of its life cycle which is necessary for HIV replication and proliferation.

The invention, it is believed, makes use of nicotinamide, an inhibitor of ADP ribosylation, to reverse the equilibrium of the NAD depletion reaction and thus suppress the metabolic depletion of niacin, the NAD precursor, thereby inhibiting the HIV replication. Furthermore, others have found that AZT, DDI and DDC prevent HIV replication at a pre transcriptional state (i.e., reverse transcription) and, thus, the combined use of nicotinamide, a post transcriptional inhibitor, with other HIV inhibitors is believed suitable for the effective therapeutic treatment of AIDS.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph which shows that cell free HIV production from HIV infected cells is inhibited in dose dependent fashion by increasing millimolar concentrations of nicotinamide measured by the Reverse Transcriptase Assay.

Fig. 2 is a graph which shows that the surviving cells of Fig. 1 exhibited a CD4 marker with increased concentrations of nicotinamide measured by flow cytometry to study the surface markers of the surviving lymphocytes.

Fig. 3 is a graph which shows that inhibition of cell free HIV production from HIV infected cells is specific for nicotinamide and no inhibition of HIV is demonstrated by nicotinic acid, the other form of niacin, or thiamine, another B-complex vitamin measured by the Reverse Transcriptase Assay.

Fig. 4 is a graphs which shows that nicotinamide inhibition of HIV is occurring at the post transcriptional level demonstrated by chronically HIV infected U1 cells with interleukin induced gene expression.

Fig. 5 is a graph which shows that nicotinamide does not inhibit the activity of HIV 1 Reverse Transcriptase.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a method for treatment of HIV and its clinical effects of the three D's by administering nicotinamide to an HIV infected patient in an amount effective to elicit a therapeutic response through post-transcriptional inhibition of HIV. It is preferred to administer nicotinamide in "pharmacologic doses", i.e., greater than 100 times the recommended daily allowance (RDA), or in an amount of 1 to 5 grams per day. The nicotinamide preferably may be administered in equally divided doses taken approximately every eight hours.

The immediate or short term goal of the invention is to administer sufficient nicotinamide so as to obviate the 3 D symptoms exhibited by a patient. Of course, nicotinamide therapy can be continued indefinitely thereafter to maintain this state. Routine evaluation of red blood cell NAD levels could be made to determine whether sufficient nicotinamide is administered to satisfy established norms.

Another clinical evaluation to be made routinely to confirm administration of sufficient nicotinamide to inhibit HIV is the measurement of viable CD4 lymphocytes. A patient CD4 level of 500 or greater demonstrates HIV inhibition, and is a goal of this invention.

Nicotinamide therapy at dosage levels sufficient to satisfy this invention is safe as well as effective, and is

desired because of its low side effect profile, and its high therapeutic index.

The nicotinamide may be administered orally, parenterally and rectally, and with any pharmaceutically accepted adjuvant or carrier. Laboratory and clinical studies to date have demonstrated utility only for the compound known as nicotinamide. It is postulated however, that nicotinic acid could be found in future clinical studies to produce the desired therapeutic effect.

This invention will be hereinafter explained more in detail by way of examples. However, these examples should not be construed to limit the scope of the invention and are to be understood merely for the purpose of illustration.

EXAMPLE 1 - HIV Infection In Human Lymphocytes

Heparinized blood from a HIV seronegative donor was centrifuged using the Ficoll Hypaque gradient technique to isolate the peripheral blood lymphocytes. The lymphocytes were then suspended in culture media (RPMI-1640 WITH 10% FBS) at a concentration of 2 million cells/ml. The cells were incubated with 5µg/ml of PHA for 72 hours. Following PHA stimulation the cells were divided into 4 culture flasks containing 10 million cells and 100 ng (p24 equivalent) of HIV-Z6. Three hours post infection the cells were washed with phosphate buffered saline, and then resuspended in 10ml of culture media (final concentration of 1 million cells/ml) with 2.5µ/ml of IL2. At

the time of resuspension three of the flasks received varied amounts of nicotinamide (NAm) and one flask was maintained as positive control (without NAm).

Post infection aliquiots were taken every 2 days for measurement to virus production. The samples were stored at -20C until the completion of the experiment and then the RT assay was performed on all samples to quantitate the virus production at 2, 4, 6, 8, and 10 days post infection.

In Fig. 1 the abscissa indicates the time post infection in days, and the ordinate indicates the amount of cell free virus produced in each culture. The results demonstrate a dose dependent inhibition of HIV production with 5mM, 10mM and 15mM concentration of NAm.

EXAMPLE 2 - Lymphocytes Subsets In Active HIV Injection

The lymphocytes from Example 1 were taken from culture on post infection day 11 and subject to flow cytometry (Becton Dickinson Lysys II Version 1.0) in an effort to demonstrate the percentage of viable cells in each culture which had detectable CD4 and CD8 surface markers.

A productive HIV infection will decrease the percentage of lymphocytes bearing CD4 marker by two means: cytotoxicity to infected cells and downregulation of the marker in infected cells. In culture the percentage will steadily decrease over time from an original percentage of approximately 60-70%. The loss of CD4+ lymphocytes in patients is the hallmark of

advancing immunosuppression and clinical decline in HIV infection.

In Fig. 2 the abscissa marks the concentrations of NAm added to each culture and the ordinate indicates the percentage of living cells which fluoresced positive for CD4, CD8 or neither (0). Note that a small percentage of cells can fluoresce positive for both CD4 and CD8 and thereby make the total in the column exceed 100%. The results demonstrate that the addition of nicotinamide not only inhibited cell free virus production but also preserved CD4+ lymphocytes in a dose dependant manner.

EXAMPLE 3 - HIV Infection In Human Lymphocytes

The infection of peripheral blood lymphocytes from seronegative donors was carried of in this experiment in a manner identical to that described in Example 1. In this example, however, at the time of resuspension in culture media containing IL2, the four culture flasks were treated with: 5mM nicotinamide, 5mM nicotinic acid, 5mM thiamine, or no addition (positive control).

In Fig. 3 the abscissa indicates the time post infection in days and the ordinate indicates the amount of cell free virus produced in each culture. The results indicate that the inhibition of HIV by nicotinamide cannot be generalized to nicotinic acid (another form of niacin) nor to thiamine (another B-complex vitamin) under these conditions.

EXAMPLE 4 - Post Transcriptional Inhibition of HIV

The U1 cell line, which is chronically infected with two proviral copies of HIV, was used in an experiment taking 4 culture flasks containing U1 cells at a concentration of $1x10^6$/ml in 5ml of culture media (RPMI 1640 containing 10% FBS). Three flasks were then either treated with the post transcriptional stimulator IL6 [10µ/ml] and/or NAm [5mM]; with the fourth flask acting as a negative control. Aliquoits were taken at days 4 and 8 post stimulation and were stored at -20C until they were subject to RT Assay.

In Fig. 4 the abscissa indicates the number of days post stimulation, and the ordinate indicates the amount of HIV produced as measured by RT assay. The results indicate that while NAm alone has an inhibitory effect on the low level constitutive production of HIV in the negative control; it has a profound inhibitory effect (IL6/5mM NAm) on the post transcriptional stimulation of HIV production in the positive control (IL6 alone).

EXAMPLE 5 - Reverse Transcriptase Activity

The effect to NAm on the activity of the HIV reverse transcriptase enzyme was assessed in a cell free system to see if this inhibitor also has direct inhibitory effects on this viral enzymes activity.

The reverse transcriptase assay was run in three wells. Each well contained 50µl of stock solution made of: 10 ml of 1M

Tris(pH7.8), 5 ml of 3M KCI, 4 ml of 0.1M DTT, 6.6ml of 0.15M MgCl, 10ml of 100µg/ml PolyA, 10 ml of 31.5 µ/ml oligo dT, 200µl of 10µCi/ml dttP, 5ml of 2% NP-40 and 149.2 ml of ddH2o. Two of the wells then had 50 ng (p24 equivalent) of HIV-HXB2 added to them and the experimental well had NAm added to a final concentration of [5mM].

In Fig. 5 the positive control (HIV and Solution) shows no statistical difference from the well with 5mM NAm added, though both are significantly different from the background activity shown in the negative control. This demonstrates the lack of significant inhibition of HIV reverse transcriptase by NAm which further supports the discovery that the inhibitory effect of NAm on HIV is post transcriptional and therefore at a site separate from that where it is taught that compounds currently used to inhibit HIV in infected patients (i.e., -AZT, DDI, and DDC) exert their therapeutic effect.

Studies completed to date have not been sufficient to determine whether the combined nicotinamide (post transcriptional) and AZT, DDI and DDC (RT) therapy results in a cumulative or greater then cumulative effect. It is possible, however, that reduced amounts of either the post transcriptional or RT effective compounds could be administered and achieve the desired therapeutic response or that a greater than expected therapeutic response could be obtained with the

administration of usual amounts of the post transcriptional and RT effective compounds.

What is claimed is:

1. A method of inhibiting human immunodeficiency virus (HIV) which comprises:

administering to an HIV infected patient a therapeutically effective amount of a post transcriptional inhibitor of HIV.

2. A method of inhibiting human immunodeficiency virus (HIV) which comprises:

administering nicotinamide to an HIV infected patient in an amount effective to elicit a therapeutic response through post transcriptional inhibition of HIV.

3. The method of claim 2 wherein said nicotinamide is administered in an amount of from about 1 gram to about 5 grams per day.

4. The method of claim 3 wherein the nicotinamide is administered until 3 D symptoms exhibited by a patient are eliminated.

5. The method of claim 3 wherein the nicotinamide is administered in an amount and for a time sufficient to achieve a patient CD4 level of 500 or greater.

6. The method of inhibiting HIV of claim 3, wherein nicotinamide is administered orally, parenterally and rectally.

7. The method of inhibiting HIV which comprises:

administering to an HIV infected patient a therapeutically effective amount of a post transcriptional inhibitor of HIV in combination with a RT inhibitor of HIV.

8. The method of claim 7 wherein the post transcriptional inhibitor is nicotinamide and the RT inhibitor is a member selected from the group comprising AZT, DDI, DDC and other RT HIV inhibitors.

ABSTRACT OF THE DISCLOSURE

This invention relates to a method of inhibiting human immunodeficiency virus (HIV) which comprises administering a therapeutically effective amount of nicotinamide to HIV infected patients. This invention further relates to post transcriptional inhibition of HIV replication in infected and uninfected cells of a patient with HIV.

Figure 1

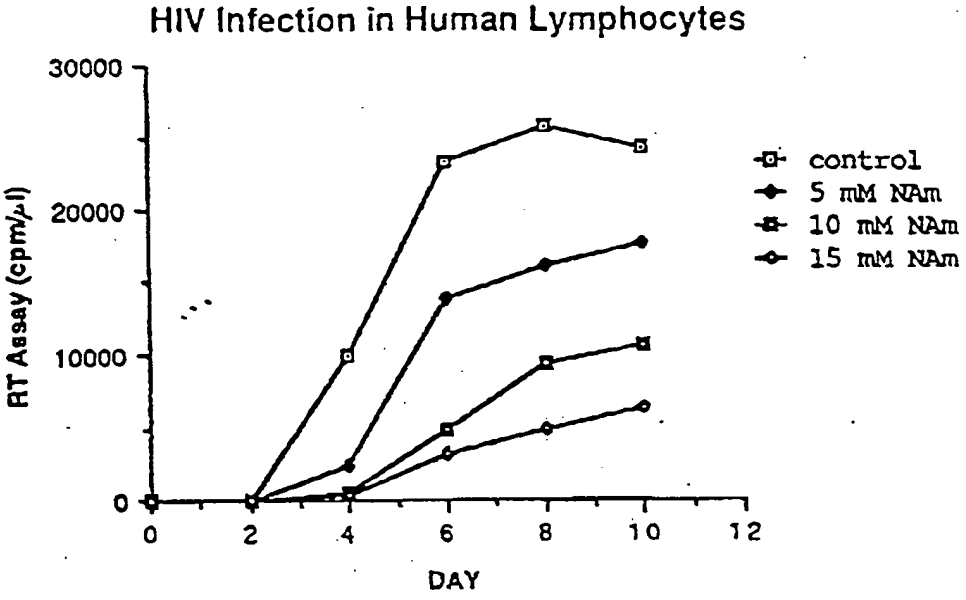

HIV Infection in Human Lymphocytes
RT Assay (cpm/μl)
30000
20000
10000
0
0
2
4
6
8
10
12
DAY
control
5 mM NAm
10 mM NAm
15 mM NAm

Figure 2

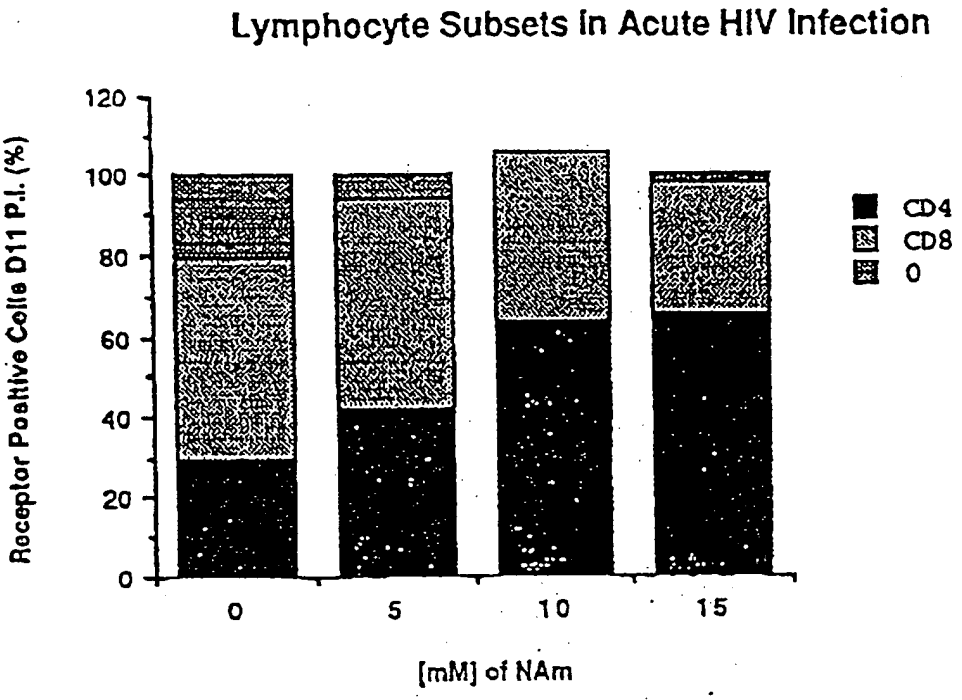

Lymphocyte Subsets in Acute HIV Infection
Receptor Positive Cells D11 P.I. (%)
120
100
80
60
40
20
0
0
5
10
15
[mM] of NAm
CD4
CD8
0

Figure 3

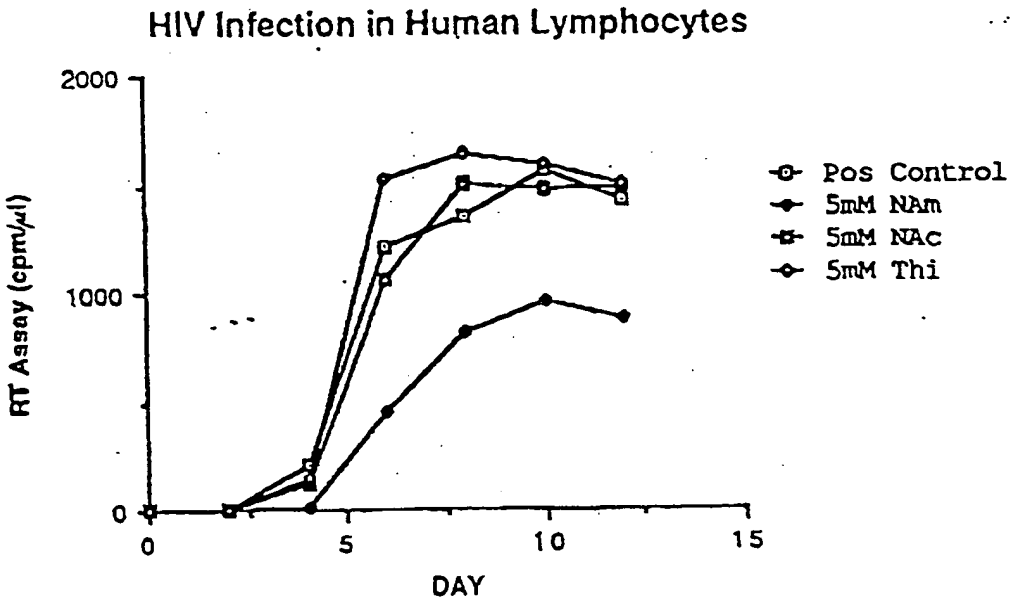

HIV Infection in Human Lymphocytes
RT Assay (cpm/μl)
2000
1000
0
0
5
10
15
DAY
Pos Control
5mM NAm
5mM NAc
5mM Thi

Figure 4

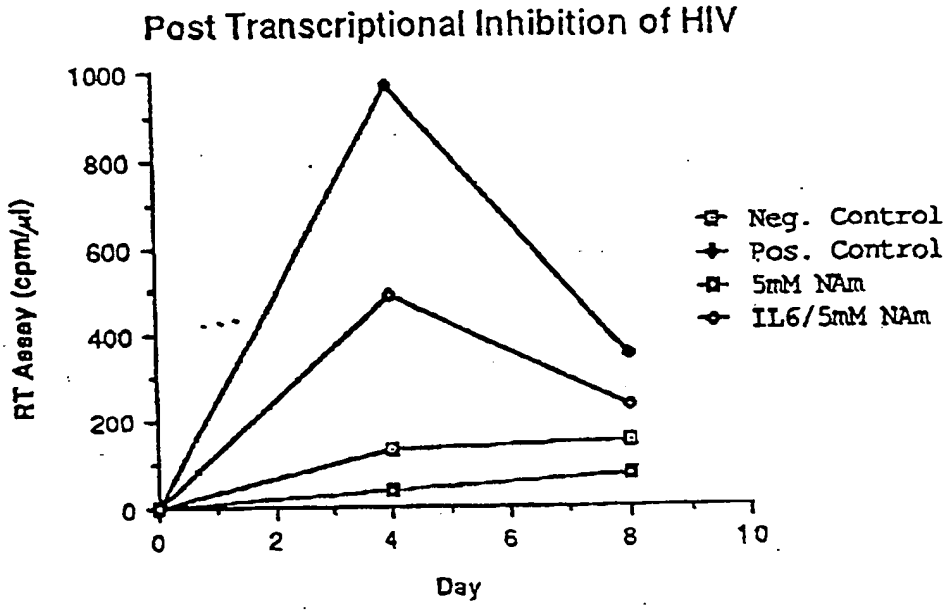
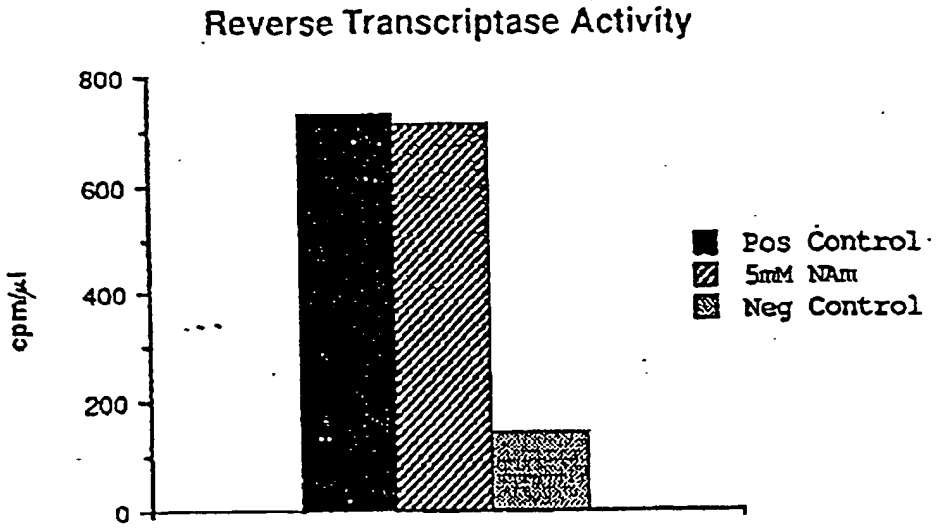

Post Transcriptional Inhibition of HIV
RT Assay (cpm/µl)
1000
800
600
400
200
0
0
2
4
6
8
10
Day
Neg. Control
Pos. Control
5mM NAm
IL6/5mM NAm

Figure 5


Reverse Transcriptase Activity
800
600
400
200
0
cpm/µl
Pos Control
5mM NAm
Neg Control